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Comparative Resistance of *Anopheles albimanus* and *Aedes aegypti* to *N,N*-Diethyl-3-methylbenzamide (Deet) and 2-Methylpiperidinyl-3-cyclohexen-1-carboxamide (AI3-37220) in Laboratory Human-Volunteer Repellent Assays

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ABSTRACT The insect repellents *N,N*-diethyl-3-methylbenzamide (Deet) and the racemate and 1*S*,2'*S* stereoisomer of 2-methylpiperidinyl-3-cyclohexene-1-carboxamide (AI3-37220) were tested against *Anopheles albimanus* Wiedemann and *Aedes aegypti* (L.) in laboratory human-volunteer assays. Estimated skin doses of Deet or racemic AI3-37220 required to reduce biting by 95% in *Ae. aegypti* were 2.3 and 3.5×10^{-2} $\mu\text{mol}/\text{cm}^2$ skin, respectively, whereas estimated doses for 95% bite reduction of *An. albimanus* in an ≈ 40 -yr-old laboratory colony established from El Salvador were 5 times higher at 12×10^{-2} $\mu\text{mol}/\text{cm}^2$ skin and $>20 \times 10^{-2}$ $\mu\text{mol}/\text{cm}^2$ skin for AI3-37220. In tests with the 1*S*,2'*S* stereoisomer of AI3-37220, a newly established colony of *An. albimanus* from Belize bit less aggressively than El Salvador *An. albimanus*. However, the Belize-derived mosquitoes were as resistant as the old El Salvador colony to repellent effects of 1*S*,2'*S* stereoisomer of 2-methylpiperidinyl-3-cyclohexene-1-carboxamide. Earlier workers surmised that usual skin doses of Deet would offer only limited protection against *An. albimanus* in the field. Our findings support this speculation, but they also indicate that doses of Deet higher than those needed for protection against *Ae. aegypti* might offer reasonable protection against *An. albimanus*. Results indicate that neither racemate nor 1*S*,2'*S* stereoisomer of 2-methylpiperidinyl-3-cyclohexene-1-carboxamide offer as much protection as Deet against *An. Albimanus*, despite being highly effective against *Ae. aegypti*.

KEY WORDS *N,N*-diethyl-3-methylbenzamide, insect repellent, (1*S*,2'*S*)-2-methylpiperidinyl-3-cyclohexen-1-carboxamide

Anopheles albimanus Wiedemann is a vector of malaria that has a wide distribution that includes lowland Middle America and the Caribbean (Belkin et al. 1970, Faran 1980). Results from field and laboratory studies of the sensitivity of *An. albimanus* to Deet have been contradictory. Deet offered high levels of protection against this species in field tests in the Panama Canal zone (Altman 1969), and Deet was more effective than all other candidate repellents in the study. How-

ever, laboratory studies by Rutledge et al. (1978) showed that among 18 species and strains tested, *An. albimanus* was the least sensitive to Deet and that this compound offered little protection against biting by this species. Schreck (1985) compiled data from 110 laboratory tests with *An. albimanus* over 10 years at the United States Department of Agriculture laboratory in Gainesville, FL. Although no statistical analysis was performed "because of wide variation in sample sizes," Schreck (1985) concluded that only limited protection against *An. albimanus* could be expected in the field if using Deet. Ostensibly, the Rutledge et al. (1978) and Shreck (1985) studies were both conducted using the same colony of *An. albimanus* from Gainesville that was established and reared continuously from insects collected in El Salvador in the early 1960s. Thus, tests showing Deet insensitivity in these two laboratory studies may have been biased because they likely used the same El Salvador inbred-laboratory stock, perhaps with a genetic predisposition for resistance to Deet. Given the potential insensitivity of *An. albimanus* to Deet, we designed an experiment using the El Salvador *An. albimanus*, comparing its sensitivity to Deet and to the comparatively newer repellent compound

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racemic 2-methylpiperidinyl-3-cyclohexene-1-carboxamide (Klun et al. 2003). As control, we concurrently tested these repellents against *Aedes aegypti* (L.), for which we have accumulated considerable experience and data (Klun, Schmidt and Debboun 2001, Klun et al. 2003). To see whether a wild population of *An. albimanus* showed a similar pattern of repellent insensitivity, we also tested a newly established colony of *An. albimanus* from Belize concurrently with the El Salvador *An. albimanus* and *Ae. aegypti* colonies against the 1S,2'S stereoisomer of 2-methylpiperidinyl-3-cyclohexene-1-carboxamide, which has been shown to be significantly more effective against *Ae. aegypti* than racemate (Klun et al. 2001).

Materials and Methods

Mosquitoes. *Ae. aegypti* (red eye Liverpool strain) and *An. albimanus* (El Salvador strain) used in the study were from colonies maintained at the Walter Reed Army Institute of Research (WRAIR). Both colonies were maintained at WRAIR for many years, and both were probably established originally at the United States Department of Agriculture Laboratory in Gainesville (Rutledge et al. 1978). A fresh colony of *An. albimanus* was established from females that were field collected 19–30 August 2002 at multiple sites in Orange Walk District, Belize. The females were given blood meals and transported to the insectary at the Uniformed Services University of the Health Sciences in Bethesda, MD. These females were used to establish a colony, which was reared for five generations. Female progeny from the original wild-caught Belize females were used in the first set of repellent tests (study 1). Because this population was derived from females not fertilized in captivity, they were considered parental stock. Females from the F4 generation were used in the second set of repellent tests (study 2). Insects were reared (Gerberg et al. 1994) by feeding larvae ground Tetramin Tropical Fish-food Flakes (Tetra Sales, Blacksburg, VA). Adults were maintained in a photoperiod of 12:12 (L:D) h at 27°C and 80% RH with a cotton pad moistened with 10% aqueous sucrose solution; they were not blood fed.

Chemicals. The repellent compounds 2-methylpiperidinyl-3-cyclohexene-1-carboxamide (AI3-37220) and *N,N*-diethyl-3-methylbenzamide (Deet) used in the tests were at least 98% pure chemically according to capillary gas-liquid chromatography. Deet and racemic AI3-37220 were obtained from Morflex, Inc. (Greensboro, NC), and the 1S,2'S stereoisomer of AI3-37220 obtained from the Chemicals Affecting Insect Behavior Laboratory where it had been synthesized previously (Klun et al. 2003). For brevity, we refer to racemic AI3-37220 and its 1S,2'S stereoisomer as 220 and SS220, respectively. Deet is a widely used arthropod repellent that is registered with the U.S. Environmental Protection Agency. The compounds 220 and SS220 have been proven to be toxicologically safe for use by humans (Snodgrass 1995, 2000; Snodgrass and Houpt 2002).

Bioassay. In conducting this research, we adhered to the guidelines established by the National Institutes of Health for tests involving human subjects, and protocols were approved by the Human-Use Review Board of the Walter Reed Army Institute of Research.

Study 1 measured the blood feeding (biting) frequency of *Ae. aegypti* and El Salvador *An. albimanus* mosquitoes in response to 0.0 (control), 2.4, 4.8, 9.6, and $19.2 \times 10^{-2} \mu\text{mol}/\text{cm}^2$ skin doses of Deet (treatments 1–5) and 220 (treatments 6–10) applied to human volunteers. Treatments 1 and 6 served as controls (skin treated with ethanol alone). Bioassays were conducted using K & D modules and methods described by Klun and Debboun (2000). A volunteer wearing short pants was seated. Using a skin-marking template and a washable-ink marker, skin areas representing five 3 by 4-cm floor openings of the K & D module were outlined on the outer, top, and inner positions of each thigh. Positions on the thigh treated with Deet or 220 were randomly selected. Locations for treatments 1–5 of Deet or treatments 6–10 of 220 within positions were randomized and labeled numerically. All treatments were pipetted onto a 4 by 5-cm rectangular area 0.5 cm outside of the template marks of the volunteers' skin in 55 μl of ethanol/treatment. Treating outside template marks assured that areas beneath each K & D module cell contained no untreated skin. Each of five adjacent cells in the K & D modules were provided with five female mosquitoes randomly selected from cages containing ≈ 200 adults. Mated nulliparous females (5–15 d old) had access only to water 24 h before testing. The K & D module was positioned with the cells aligned over the marked and treated areas of skin. Sliding doors between the cell and skin were then opened. For the next 2 min, the number of females biting (proboscis inserted into skin and/or observed blood-engorged females) within each of the cells was recorded. The trial was concluded at the end of the 2-min period by closing the sliding doors. Individual mosquitoes were scored as having either fed or not fed during a trial. The bioassays were done in a walk-in incubator (27°C and 80% RH) in ambient fluorescent light from 0730 hours to 1030 hours. Observations using *An. albimanus* were replicated 24 times with four volunteers (1,200 total mosquitoes used) and observations with *Ae. aegypti* were replicated 18 times with five volunteers (900 total mosquitoes used). Three of the six volunteers were involved in tests against both species. Mosquitoes were used for only one test and modules were washed with water and detergent after each trial.

Study 2 measured the responses of the newly established *An. albimanus* Belize colony, the El Salvador *An. albimanus* colony, and the red eye Liverpool *Ae. aegypti* colony to either an ethanol control or $2.4 \times 10^{-2} \mu\text{mol}$ of SS220/ cm^2 skin, using the same methods described above. SS220 stereoisomer was used in this test because the compound was previously demonstrated (Klun et al. 2001) to be significantly more effective against *Ae. aegypti* than 220 (racemate), and it was surmised that SS220 might likewise be more effective than the racemate against *An. albimanus*. The

SS220 dose was selected because a previous study (Klun et al. 2003) showed that 2.4×10^{-2} μmol of SS220/ cm^2 reduced *Ae. aegypti* biting by at least 80%. Pairs of module cells containing five mosquitoes of each of the three mosquito types were positioned randomly over SS220 treated skin or an area of control skin. Study 2 involved five volunteers (one was in study 1 and four were new volunteers) and 29 replicates (870 total mosquitoes used).

Statistical Methods. In study 1, we modeled the logit of the proportion of mosquitoes not biting as $(\log(p/(1-p)))$, where p is the proportion of mosquitoes not biting) to develop a dose-response curve. This standard transformation has desirable statistical properties for developing dose-response relationships by using data that are samples from a binomial distribution. We found that a straight line relationship between dose and logit (p) was obtained by taking the square root of dose. The log transformation on dose, recommended by Anonymous (1983) did not provide this straight line relationship, although it may be an effective transformation of dose for other mosquito species. Because there can be significant person-to-person differences in the number of mosquitoes biting for the same dose (Gilbert et al. 1966), we included person as a random variable (modeled as drawn from a normally distributed population) in the generalized linear mixed model we developed. Although it is conceivable that other block effects (K & D module replicate, position on thigh, day of trial) existed, a preliminary analysis of this data set (and experience with similar data from other studies) found these effects estimated as zero or near zero; thus, they were not included when modeling these data.

We used PROC NLMixed (SAS Institute 1999) to estimate the parameters of the model (here written for a specific species and compound, so notation for these is suppressed), $\log(\pi_{ij}/(1-\pi_{ij})) = \beta_0 + \beta_1 D_i + V_j$, where π_{ij} is the true proportion of nonbiting mosquitoes for the i th dose level (D_i) and the j th volunteer (V_j). The data for both species and both compounds were modeled jointly, by using additional parameters, so that t -tests on these parameters could be made, e.g., to test whether the species' dose-response curves differed in intercept or slope.

The estimated model parameters were used to create a dose-response model for the two species for each of the compounds and to estimate 95% fiducial limits (Draper and Smith 1981) for the dose required to obtain 95% efficacy (defined as 95% of the mosquitoes not biting) against *Ae. aegypti* (Deet and racemate 220) and *An. albimanus* (Deet only). A fiducial interval is the confidence interval on what is ordinarily the independent variable when making predictions by using what is ordinarily the dependent variable, by using inverse regression. In this experiment, dose (independent variable) was first used to estimate the proportion of mosquitoes not biting (dependent variable). Results from this regression were then used to estimate, for 95% of mosquitoes not biting, the smallest interval (the fiducial interval) that would contain the corresponding true dose 95% of the time.

The data from study 2, by using SS220 against *Ae. aegypti*, and Belizian and El Salvadorian colonies of *An. albimanus*, were treated in a manner similar to those from study 1. Because only a single dose of 2.4×10^{-2} μmol of SS220/ cm^2 skin was used, the statistical model (for a specific species and compound) reduces to $\log(\pi_{ij}/(1-\pi_{ij})) = \beta_0 + \tau_i + V_j$, with τ_i representing the effect of the repellent (versus control, $i = 0, 1$) and other symbols as defined above. Thus, any differences between populations and compounds would emerge as differences in intercepts (β_0) and values of τ_i , tested for significance using a t -test. Point estimates of the $\beta_0 + \tau_i$ s, their standard errors, and t -values were calculated using Proc NLMixed.

Results and Discussion

In study 1, *Ae. aegypti* and *An. albimanus* did not differ significantly in their propensity to bite on untreated control skin (t -test, $df = 20$, $P = 0.13$). There were significant effects (t -test, $df = 20$, $P < 0.01$) of both compound and species on the slope of the response to dose, with Deet significantly more effective than 220, and *Ae. aegypti* significantly more sensitive to both repellents than *An. albimanus*. There was no significant interaction (t -test, $df = 20$, $P = 0.56$) between species and dose, i.e., both species responded similarly to the compounds, other than differences in their overall sensitivity to repellent.

A dose-response curve, backtransformed to the original scale (proportion repelled) is shown in Fig. 1. Point estimates for 95% efficacy are 2.3×10^{-2} $\mu\text{mol}/\text{cm}^2$ skin (*Ae. aegypti*, Deet), 3.5×10^{-2} $\mu\text{mol}/\text{cm}^2$ skin (*Ae. aegypti*, 220), and 12.0×10^{-2} $\mu\text{mol}/\text{cm}^2$ skin (*An. albimanus*, Deet). Point estimates (and fiducial intervals) for 95% efficacy were not estimated for *An. albimanus* against 220 because they exceed the highest dose used, i.e., lie outside the range of the data. The 95% fiducial interval on dose for 95% efficacy for *Ae. aegypti* was $1.4\text{--}3.1 \times 10^{-2}$ $\mu\text{mol}/\text{cm}^2$ skin for Deet and $2.3\text{--}4.6 \times 10^{-2}$ $\mu\text{mol}/\text{cm}^2$ skin for 220. This interval for *An. albimanus* for Deet is $8.7\text{--}19.3 \times 10^{-2}$ $\mu\text{mol}/\text{cm}^2$ skin. Using the inverse regression equations, we estimated that a dose of Deet that repels 95% of *Ae. aegypti* mosquitoes will only be $\approx 66\%$ effective against *An. albimanus*.

Because only six volunteers were used in study 1, the variance attributable to person-to-person differences (variance = 0.15, SE of variance estimate = 0.12, on the transformed scale) was poorly estimated. However, we felt it was important to retain this term in the model because other studies (Gilbert et al. 1966) found large and systematic differences in attractiveness to mosquitoes among the human volunteers used in their study. An additional reason for retaining the term concerns the validity of t -tests if person-to-person differences are ignored. The logit transformation of a proportion produces an approximately normally distributed variable with variance $(m\pi(1-\pi))^{-1}$ (Cox and Snell 1989). Taking π as 0.95 (true proportion of mosquitoes not biting) and m as 20 (degrees of freedom for t -tests above), the variance attributed to

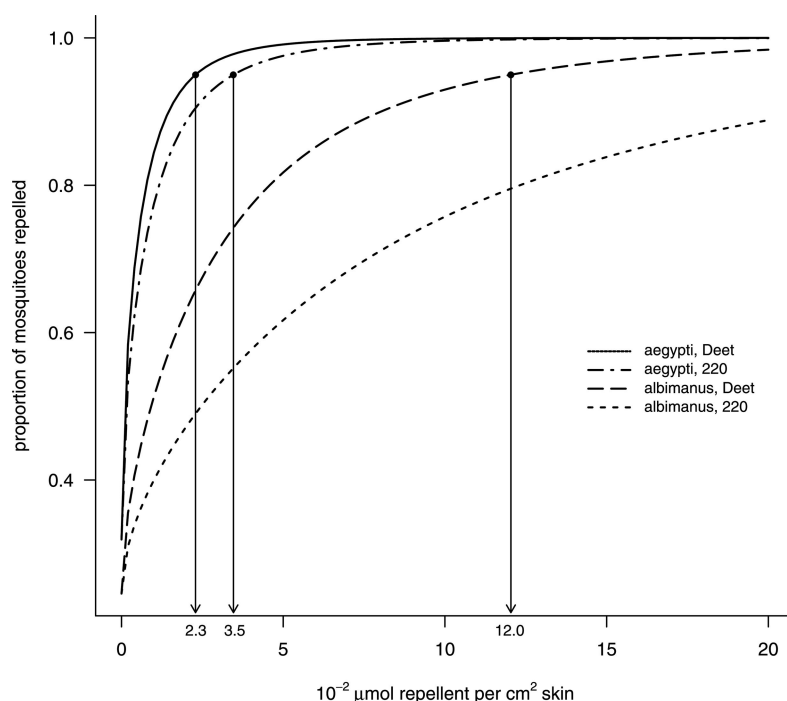


Fig. 1. Dose-response curves for *An. albimanus* (El Salvador strain) and *Ae. aegypti* (red eye Liverpool strain), for Deet and racemic AI3-37220. The arrows indicate the estimated dose necessary to prevent 95% of mosquitoes from biting.

causes other than person-to-person differences at the 95% efficacy is estimated at 1.05. If the variance due to person-to-person differences were ignored, the true variance of the residuals would be larger ($0.15 + 1.05 = 1.20$) than that assumed based on an uncontaminated binomial distribution. This would make tests of interest (species and compound differences) too liberal, (declaring differences as significant when, in fact, they are not, more than the nominal 5% of the time), similar in effect to ignoring over dispersion in χ^2 tests (Kramer and Schmidhammer 1992).

The results from study 2 are given in Table 1. The propensity to bite in the two *An. albimanus* colonies differed significantly (t -test, $df = 24$, $P < 0.01$), but biting was not significantly (t -test, $df = 24$, $P = 0.51$) affected by the presence of repellent at a dose of 2.4×10^{-2} μmol of SS220/ cm^2 skin. This is consistent with results from study 1, where, for this dose of 220, percentage of biting only decreased by an estimated 22%.

Table 1. Estimated proportions of *Ae. aegypti*, *An. albimanus* (Belize), and *An. albimanus* (El Salvador) mosquitoes not biting on treated skin (2.4×10^{-2} μmol SS220/ cm^2 skin) and untreated skin and estimated bounds on an upper and lower 95% confidence interval

Treatment	Species	Origin	Lower 95%	Estimate	Upper 95%
Control	<i>albimanus</i>	El Salvador	0.09	0.15	0.24
SS220	<i>albimanus</i>	El Salvador	0.15	0.23	0.34
Control	<i>albimanus</i>	Belize	0.58	0.69	0.79
SS220	<i>albimanus</i>	Belize	0.51	0.63	0.73
Control	<i>aegypti</i>	Liverpool	0.28	0.39	0.51
SS220	<i>aegypti</i>	Liverpool	0.86	0.93	0.96

In contrast, for this dose, biting by *Ae. aegypti* mosquitoes was suppressed by $>90\%$.

The substantial difference in biting tendency between the two *An. albimanus* colonies demonstrates that large local intraspecific variation among mosquito populations can exist. This potential variation is typically ignored when testing repellents, and is, in general, poorly understood. We have also often seen large variation in the level of biting propensity among several colonized species in other laboratory repellent studies (unpublished data) that occur from time to time with no explainable reason. Thus, a formulation that repels 95% of mosquitoes in one population at one time may be more or less effective against that same species at a different locality or time. What seems to be consistent is the relative resistance of the two *An. albimanus* populations to repellents. Thus, relative resistance to the repellents is a species rather than population characteristic. In an earlier study of Deet and SS220 against *An. stephensi* (Klun et al. 2003), there was no detectable resistance to either compound, and this is evidence that resistance seen in *An. albimanus* is not a uniform characteristic in the genus.

Although there was some difference among compounds in effectiveness, with Deet slightly more effective than racemic 220 for both mosquito species, this difference was largely overshadowed by the large species response differences to the compounds. Because protection is desired against all mosquito species a person is exposed to, an effective mosquito repellent should repel the least sensitive species most of the time. Thus, to obtain a meaningful test of repellent

compounds, trials should be conducted ideally with a variety of mosquito species and from a variety of different locations or origins, with an emphasis on those, like *An. albimanus*, that have demonstrated relative insensitivity to repellent compounds. It will be of interest to learn whether field tests of *An. albimanus* populations at various locations across Middle America reveal the same indications of repellent resistance as seen in our laboratory investigations with the mosquitoes colonized from El Salvador and Belize. In practice, formulated repellents are often applied at dosages far in excess of that used in our laboratory assays. We surmise the failure of formulated products against resistant species such as *An. albimanus* will usually be observed as dramatically reduced protection time, rather than as the complete absence of protection.

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References Cited

- Altman, R. M. 1969. Repellent tests against *Anopheles albimanus* Wiedemann in the Panama Canal zone. Mosq. News 29: 110–112.
- Anonymous. 1983. Standard test methods for laboratory testing of non-commercial mosquito repellent formulations on the skin. Standard E951-83. American Society for Testing and Materials, Philadelphia, PA.
- Belkin, J. N., S. J. Heinemann, and W. A. Page. 1970. Mosquito studies (Diptera, Culicidae). XXI. The Culicidae of Jamaica. Contr. Am. Entomol. Inst. 1: 1–458.
- Cox, D. R., and E. J. Snell. 1989. Analysis of binary data, 2nd ed. Chapman & Hall, New York.
- Draper, N. R. and H. Smith. 1981. Applied regression analysis, 2nd ed. Wiley, New York.
- Gerberg, E. J., D. P. Barnard, and R. A. Ward. 1994. Manual for mosquito rearing and experimental techniques. Am. Mosq. Control Assoc. Bull. No. 5 (revised). AMCA, Inc., Lake Charles, LA.
- Gilbert, I. H., H. K. Gouck, and N. Smith. 1966. Attractiveness of men and women to *Aedes aegypti* and relative protection time obtained with Deet. Fla. Entomol. 49: 53–66.
- Faran, M. E. 1980. Mosquito studies (Diptera, Culicidae) XXXIV. A revision of the *albimanus* section of the subgenus *Nyssorhynchus* of *Anopheles*. Contrib. Am. Entomol. Inst. (Ann Arbor) 15: 1–215.
- Klun, J., A. Khirman, A. Margaryan, M. Kramer, and M. Debboun. 2003. Synthesis and repellent efficacy of a new chiral piperidine analog: comparison with Deet and Bayrepel activity in Human-volunteer laboratory assays against *Aedes aegypti* and *Anopheles stephensi*. J. Med. Entomol. 40: 293–299.
- Klun, J. A., and M. Debboun. 2000. A new module for quantitative evaluation of repellent efficacy using human subjects. J. Med. Entomol. 37: 177–181.
- Klun, J. A., W. F. Schmidt, and M. Debboun. 2001. Stereochemical effects in an insect repellent. J. Med. Entomol. 38: 809–812.
- Kramer, M., and J. Schmidhammer. 1992. The chi-squared statistic in ethology: use and misuse. Anim. Behav. 44: 833–841.
- Rutledge, C. C., M. A. Moussa, C. A. Lowee, and R. K. Sofield. 1978. Comparative sensitivity of mosquito species and strains to the repellent diethyl toluamide. J. Med. Entomol. 14: 536–541.
- SAS Institute. 1999. SAS/STAT user's guide, version 8. SAS Institute, Cary, NC.
- Schreck, C. E. 1985. The status of DEET (N,N-diethyl-M-toluamide) as a repellent for *Anopheles albimanus*. J. Am. Mosq. Control Assoc. 1: 98–100.
- Snodgrass, H. L. 1995. Toxicology study no. 75-51-Y2Z2-95, The acute toxicity of a mixture of the insect repellents Deet and AI3-37220, May 1995. U.S. Army Center for Health Promotion and Preventative Medicine, Aberdeen Proving Ground, MD.
- Snodgrass, H. L. 2000. Toxicology study no. 85MA2373-01, Skin sensitization and cumulative irritancy of the insect repellents Deet and AI3-37220 in humans. November 2000. U.S. Army Center for Health Promotion and Preventative Medicine, Aberdeen Proving Ground, MD.
- Snodgrass, H. L., and J. T. Houpt. 2002. Toxicology study no. 85-XC-3929-02, protocol no. 3929-23-01-07-02, The acute dermal toxicity of the repellent SS-220 in guinea pigs, 17 September–1 October 2002. U.S. Army Center for Health Promotion and Preventative Medicine, Aberdeen Proving Ground, MD.

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